Expression Quantitative Trait Loci (eQTL) Analysis Of *Gluteus Medius* Muscle In Commercial Duroc Pigs

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Introduction

In the last couple of decades, the pig industry has strived to better define the genetic basis of meat quality traits in pigs. In this sense, more than 1400 QTL that influence traits dealing with lipid metabolism and meat quality have been identified. Expression quantitative trait loci (eQTL) mapping is a powerful tool to identify genetic variation with regulatory effects on mRNA levels. In the present study, we have carried out an eQTL study in order to detect pig genome regions regulating levels of skeletal muscle mRNA expression and associated with lipid metabolism and meat quality traits.

Material and methods

Animal material. An experimental pig population (350 castrated males) distributed in five half-sibs families and four contemporary groups was generated from a commercial Duroc line. A number of phenotypes dealing with fatness, serum lipid levels, and intramuscular fat content and composition were recorded. See Gallardo et al. (2008) for further details.

Expression data and normalization. We have analyzed the global mRNA expression profile of *gluteus medius* muscle samples obtained from 104 individuals belonging to two groups (52 individuals per group) with divergent lipid metabolic profiles. Groups were established on the basis of multivariate analyses for several lipid deposition traits. Expression data were obtained using *GeneChip Porcine Genome*® arrays *(Affymetrix)*. Data was normalized using the gcRMA algorithm.

eQTL analyses. A genome-wide eQTL scan was carried out for 6139 *Affymetrix* probes, more than 20% of the probes displayed expression values over ± 1.5 times the median expression of all arrays. The eQTL analyses was performed for each probe using a panel of 110 microsatellites using the *GridQTL* software (http://www.gridqtl.org.uk/)(Seaton et al. 2006) with the following statistical model:

$$y_{ijk} = \mu + b_i + l_j + \sum_{sire=1}^{5} \alpha_{sire} p_{k(sire)} + e_{ijk}$$

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Where, y_{ijk} is the expression data of individual k; b_i is the effect of i^{th} contemporary group of fattening (4 levels); l_j is the laboratory j effect (2 levels); α and p_k are, respectively, the regression coefficient (mean allele substitution effect) and the probability of individual k inheriting a given allele from its common parent. Genome-wide significance thresholds for the F-values (eQTL model *vs* no eQTL model) were approximated with Bonferroni correction.

Reference assembly analysis. For those target probes with significant eQTL the affymetrix sus scrofa probe-sets (11 probes per probe-set) were analyzed. The probes were assembled to the Ensemble porcine genome (ftp://ftp.ensembl.org/pub/current_embl/sus_scrofa/) using CLCBio workbench software. The quality of matches of all significant probes was analyzed, and probes were classified as uniquely mapped and non-specifically mapped probes.

Results and discussion

In the whole-genome scan carried out for 6139 transcripts, a total of 613 eQTL at genome-wide significance level were identified distributed across all the pig genome. There was an unequal distribution across the 18 autosomes resulting in SSC5 and SSC3 having an enrichment of eQTL (107 and 74 respectively), whereas only 12 and 4 eQTL were observed on SSC4 and SSC11, respectively (Figure 1).

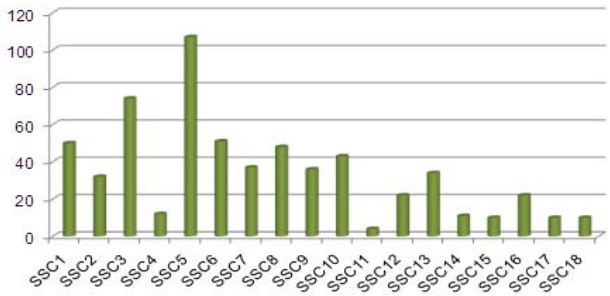

Figure 1: Number of eQTL detected at genome-wide level in each chromosome.

Results from the analysis of probe-sets on the reference assembly using CLCBio workbench software allowed mapping 478 out of 613 target probes/genes that showed significant eQTL. Most of them were uniquely mapped probes (478) while a small fraction was non-specifically mapped probes (12). We detected a high number of unmapped probes (123) due to the incomplete annotation of porcine genome available at the moment.

From the 478 mapped probes, only 63 showed a *cis*-acting eQTL, whereas the rest were *trans*-acting eQTL (Figure 2). It is assumed that *cis*-eQTL, i.e. eQTL physically close to the target transcript/gene, are caused by polymorphisms in regulatory regions of the gene. *Trans*-eQTL, i.e. located far from the target transcript/gene, are generally transcription factors or microRNAs.

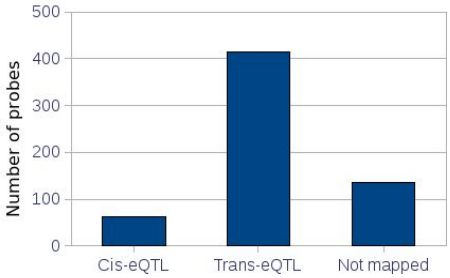

Figure 2: Mapping results for *cis*- and *trans*-acting eQTL.

The binding specificity of the probe-sets was analyzed according to the number of individual probes in each probe-set assembling to a specific region. A probe-set was classified of high quality when more than 6 out of 11 probes assembled to a unique region. Most of the *cis*- and *trans*-eQTL (94 and 95%, respectively) were targeted with high quality probes (Table 1). We discarded all low quality probe-sets from further analysis.

Table 1: Number of high and low quality porcine affymetrix probes mapped in *cis* and *trans* position.

	High quality probes	Low quality probes	Number of probes
***cis*-eQTL**	59	4	63
***trans*-eQTL**	396	19	415

The annotation and functional classification of the target genes confirmed that a number of the eQTL affected the expression of probes related to fat deposition, lipid metabolism and muscle development (Figure 3). Comparing the positions of these eQTL with QTL previously identified for intramuscular fat content (IMF) and composition revealed 96 common linkage region between QTL previously identified and eQTL. Most of them, were regions involving *trans*-acting eQTL (81) while, 15 were *cis*-acting eQTL located in SSC1, SSC3, SSC6, SSC7, SSC8, SSC14 and SSC18 (Table 2) (Gallardo et al. 2008; Solé et al., 2009).

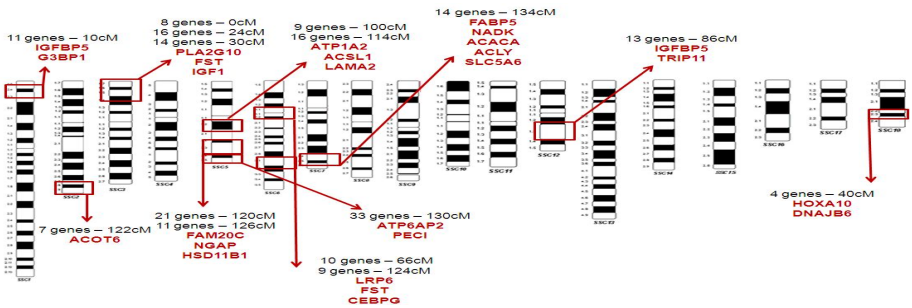

Figure 3: Main eQTL regions and some of the probes related with lipid metabolism and muscle development regulated by them.

Table 2: Common linkage region between QTL previously identified and eQTL.

Gene	Chromosome	eQTL location	Trait QTL	QTL location
ABAT	SSC03	010cM	IMFcovBFT	15cM
ABAT	SSC03	010cM	Vaccenic FA	15cM
ANXA8L1	SSC14	044cM	Palmitoleic FA	50cM
DNAJB6	SSC18	040cM	Palmitic FA	35-37cM
GSPT1	SSC03	010cM	IMFcovBFT	15cM
GSPT1	SSC03	010cM	Vaccenic FA	15cM
HOXA10	SSC18	040cM	Palmitic FA	35cM
IL12RB2	SSC06	092cM	IMFcovLW	92cM
LCMT1	SSC03	024cM	IMFcovLW	28cM
MEPCE	SSC03	024cM	IMFcovLW	28cM
NEDD4L	SSC01	064cM	IMFcovLW	62cM
NUDT6	SSC08	076cM	Myristic FA	76cM
PAFAH1B3	SSC06	070cM	Vaccenic FA	73cM
PPP1CB	SSC03	024cM	IMFcovLW	28cM
SLA-1	SSC07	072cM	Palmitoleic FA	70cM

Conclusion

In the present work we carried out a genome-wide eQTL analysis of *gluteus medius* skeletal muscle tissue from animals divergently selected for lipid metabolism profile. We detected 613 eQTL unequally distributed across the pig genome. The functional classification of the target genes showed that a number of these eQTL affected the expression of genes related to fat deposition, lipid metabolism and muscle development. Moreover, 63 of this eQTL were located in *cis* position, some of them co-localizing in genome regions where QTL for serum lipid levels and intramuscular fat content and composition had been previously described. These results suggested a number of candidate genes that may be responsible for regulating lipid metabolism and meat quality in the studied commercial Duroc pig population.

References

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